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Applicants: Breton et al.
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PHOTOPROTECTION OF THE SKIN
Art Unit: Unknown
Examiner: Unknown
Docket No.: 112701-434

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

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Applicants are respectfully enclosing the certified copy of the priority document for which priority is claimed for the above-identified application under 35 U.S.C. §119. Specifically, the document enclosed is:

Document No.	Country	Date
02075703.5	Europe	February 21, 2002

Respectfully submitted,

BELL, BOYD & LLOYD LLC



BY _____

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Dated: October 27, 2004

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The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

02075703.5

**PRIORITY
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R C van Dijk



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Anmelder/Applicant(s)/Demandeur(s):

SOCIETE DES PRODUITS NESTLE S.A.
Case postale 353
1800 Vevey
SUISSE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
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Orally administrable composition for the photoprotection of the skin

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s)
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AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR

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Orally administrable composition for the photoprotection of the skin 

Field of the invention

5 The present invention relates to orally administrable composition or pharmaceutical compositions, or cosmetical compositions, for the protection of the skin against negative effects from the environment, in particular exposure to solar radiation, which is orally administrable, and a method to improve the photoprotection of the skin.

10

Background of the Invention

15 The continuous decrease of the atmosphere's ozone layer with the concurrent increase of ultraviolet radiation reaching the planet's surface has attracted a great deal of interest in its potential consequence on human health. Although exposure to ultraviolet radiation is needed for humans to produce vitamin D, growing evidence suggests that extensive exposure to sun-light, in particular to ultraviolet radiation, causes a variety of problems in the skin, including induction of certain skin cancers and induction of accelerated skin ageing.

20

In addition to these established health concerns, research has also provided evidence suggesting that exposure to ultraviolet radiation may negatively affect a variety of immune responses in living beings both locally, within the UV-irradiated skin, and also systemically, i.e. at sites distant from the irradiated skin.

25

It is thus important to alleviate the detrimental effects of ultraviolet radiation on the skin, and also prevent the development of erythema, oedema and/or flaking or scaling (hyperkeratosis) of the skin.

30

In the art, there have been several attempts, such as by using sunscreens or other particular pharmacological agents.

In J. Invest. Dermatol., 97 (1991), 624-628 it is reported that topical application of ultraviolet radiation-absorbing compounds (sunscreens) is effective in preventing ultraviolet radiation-induced erythema and edema but cannot prevent UV-light induced immuno-suppression. This finding was confirmed by several other studies,
5 according to which sunscreens seems to prevent inflammation or irritation but do not provide complete prophylactic protection against the immuno-suppressive effects of ultraviolet radiation..

On the other hand, In FR 2698 268 (L'Oreal) an orally administrable composition
10 comprising a combination of at least one amino-acid, salt of copper and a mix of vitamins has been shown to protect the skin against ultraviolet radiation.

However, there is still a need in the art for an orally administrable composition,
15 which is capable to improve and/or reinforce the photoprotective function of the skin.

15 **Summary of the invention**

Accordingly, in a first aspect the present invention aims to provide an orally
20 administrable composition for the photoprotection of the skin which comprises a photoprotecting effective amount of i) at least one probiotic lactic acid bacterium or a culture supernatant thereof, and ii) at least one carotenoid or derivative, included into an orally acceptable carrier.

The present invention further relates to the use of a photoprotecting effective amount
25 of at least one probiotic lactic acid bacterium or a culture supernatant thereof and at least one carotenoid, included into an orally acceptable carrier for preparing an orally administrable composition for protecting the skin against solar radiations such as ultraviolet and all related skin disorders, such as erythema, inflammation, sun burn, barrier function, photoageing, alteration of the immune system, for example.

30

In a last aspect, the invention relates to a method for improving the photoprotective function of the skin, which comprises the step of orally administering to the individual a composition comprising a photoprotecting effective amount of i) at least one probiotic lactic acid bacterium or a culture supernatant thereof, and ii) at least 5 one carotenoid or derivative, in an orally acceptable carrier.

The combination according to the present invention has a particular beneficial effect on skin protection and coloration of the skin that helps to reduce the effects of solar radiation-related stress on skin.

10

Detailed Description of the Invention

Within the following description, "NCC" designates Nestlé Culture Collection (Nestlé Research Center, Vers-chez-les-Blanc, Lausanne, Switzerland). The term 15 "photoprotection" is used to describe attempt to block or reduce the adverse clinical, histological and immunological effects of solar radiation exposure on the skin.

According to the present invention, the subject compositions comprise, as the active agents therefor, combinatory immixture of at least one probiotic lactic acid bacterium 20 or a culture supernatant thereof, and at least one carotenoid or derivative.

Indeed, it has now surprisingly and unexpectedly been determined that admixture of these two very specific constituents elicits an enhanced effect or response in respect 25 of the photoprotection of the skin.

25

Probiotics are non-pathogenic and non-toxigenic organisms that survive passage through the stomach and small intestine. Upon continuous ingestion by the host they eventually may colonize the gut to a substantial extent thus competing with other potentially pathogenic bacteria for nutrients and/or attachment sites on the gastrointestinal wall and reducing their numbers and reducing or preventing infections. 30 Until now a number of different probiotic microorganisms have been found, which

all are reported to exert their effect in the gut via the production of toxins, metabolic by-products, short chain fatty acids and the like.

- 5 It has now been shown that probiotics do also exert an effect in an individual's body at a location distant from the region in which they colonize it. And particularly, it has been surprisingly found that a composition having a synergistic photoprotective effect on the skin may be obtained by combining into an orally acceptable carrier, a probiotic microorganism and an active compound such as carotenoid.
- 10 In a preferred embodiment, the probiotic to be included into the carrier is selected from the group consisting of lactic acid bacteria, in particular Lactobacilli and/or Bifidobacteria and are more preferably *Lactobacillus johnsonii*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Lactobacillus casei* or *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium animalis*, *Bifidobacterium infantis*, *Bifidobacterium adolescentis*, *Bifidobacterium pseudocatenulatum*, or a mixture thereof.
- 15

According to a most preferred embodiment the strains *Lactobacillus johnsonii* NCC 533, *Lactobacillus paracasei* NCC 2461, *Bifidobacterium adolescentis* NCC 251 and *Bifidobacterium longum* NCC 490 were deposited by way of an example, under the Budapest Treaty with the Institut Pasteur (28 rue du Docteur Roux, F-75024 Paris cédex 15) on 30.06.92, 12.01.99, 15.04.99 and 15.03.99, respectively and under the deposit number CNCM I-1225, CNCM I-2116, CNCM I-2168 and CNCM I-2170, respectively.

- 25 The strain of *Bifidobacterium lactis* (ATCC27536) provided by Hansen (Chr. Hansen A/S, 10-12 Boege Alle, P.O. Box 407, DK-2970 Hoersholm, Danemark) can also be used.
- 30 The probiotic microorganism according to the present invention may be included in a live form, dead form, semi-active or in deactivated form and fragments or fractions

- originating from the microorganism either live or dead e.g. as a lyophilized powder. Also culture supernatants of the microorganisms may be included in the products, optionally in concentrated form. It may also be included in an encapsulated form.
- 5 When using a supernatant of a probiotic's culture the supernatant may be used as such or may be subjected to one or more purification steps prior to inclusion into the product, so as to concentrate or isolate the active ingredient (s) /metabolite (s). Method and techniques for purifying compounds and detecting the activity thereof in the fractions obtained are well known to the skilled person.
- 10 The probiotic lactic acid bacteria may be present in the carrier in an amount of at least 10^5 cfu/g of carrier and preferably from about 10^5 to 10^{15} cfu/ g of orally acceptable carrier, and more preferably from 10^7 to 10^{12} cfu/g of orally acceptable carrier.
- 15 The carotenoid may be a carotenoid with or without provitamin A activity. It may be β -carotene, γ -carotene, α -carotene, lycopene, zeaxanthine and luteine, or a mixture thereof. The carotenoid may be from synthetic or natural origin or contained in a natural extract. When the carotenoid is from natural origin, it is preferably obtained from plant material, in which the plant is grown in-vivo or in-vitro. Method for extracting the carotenoids is well known in the art. The carotenoid may be present in the carrier in an amount of from $10^{-12}\%$ to 20% by weight and preferably from 0,00001 mg to 50 mg/day and more preferably from 0.001mg to 30mg/day.
- 20
- 25 A mixture of a plurality of lactic acid bacteria or carotenoids may also be used.
- The carrier may be any food or pharmaceutical product, or a nutritional supplement for oral administration or a composition for oral administration, wherein the probiotic microorganism and the carotenoid may be included. Examples for food or pharmaceuticals carriers are milk, yoghurt, curd, cheese, fermented milks, milk based
- 30 fermented products, ice-creams, fermented cereal based products, milk based powders, infant formulae or tablets, liquid suspensions, dried oral supplement, wet

oral supplement, dry-tube-feeding. The nutritionally supplement for oral administration may be in capsules, soft capsules, tablets, pastes or pastilles, gums, or drinkable solutions or emulsions. Methods for preparing the carrier are common knowledge.

5

The composition according to the invention may further comprise bioactive molecules or yeast extracts, for example. In a preferred embodiment, the yeast is any food-grade yeast selected from the group consisting of Ascomycotina or Deuteromycotina. In a preferred embodiment, the yeast may be selected from the 10 group consisting of *Debaryomyces*, *Kluyveromyces*, *Saccharomyces*, *Yarrowia*, *Zygosaccharomyces*, *Candida* and *Rhodotorula*, and more preferably *Saccharomyces caerevisae* (baker's yeast).

Such yeast may be used in the form of dried or lyophilized extracts. It may be present 15 in the carrier in an amount of at least 10^5 cfu/ g of orally acceptable carrier, preferably from about 10^5 to 10^{15} cfu/ g of orally acceptable carrier, and more preferably from 10^7 to 10^{12} cfu/ g of orally acceptable carrier, said amount depending on the nature and activity of the particular yeast.

20 The composition according to the invention may also comprise usual excipients, in particular sweeteners, flavouring agents or preservatives.

The composition according to the invention provides a surprising and synergistic protective and preventive effect of the skin.

25

Accordingly, in another aspect, the invention relates to a method for improving the photoprotective function of the skin, which comprises the step of orally administering to an individual a composition comprising a photoprotecting effective amount of i) at least one probiotic lactic acid bacterium or a culture supernatant 30 thereof, and ii) at least one carotenoid or derivative, in an orally acceptable carrier.

The amount of the composition to be consumed by the individual will depend on the desirable effect. However, an amount of the composition to provide a daily amount of about 10^5 to 10^{15} organisms, which organism may be alive or dead, and from 0,00001 mg to 50 mg of carotenoids, would usually be adequate.

5

The composition is administered to an individual before or during the exposure to ultraviolet radiation, in particular exposure to sun. When the exposure period is foreseeable, it is desirable to start the consumption before the exposure and preferably 1 to 2 months before, and to prolong consumption during exposure.

10

The following examples are given by way of illustration only and in no way should be construed as limiting the subject matter of the present application. All percentages are given by weight unless otherwise indicated.

15 **Examples**

Example 1

A photoprotective daily orally administrable composition is prepared as follows:

20 β-carotene (Roche) 4.7 mg

Latobacillus CNCM I-1225 dry mix(containing 1.10^8 to 1.10^9 organisms) 50 mg

Glucidex IT 19 (maltodextrin powder) QSP 500 mg

The composition is administered to the individual in an amount of 2x500 mg daily,
25 which provides a protective and preventive effect of the skin.

Example 2

A photoprotective daily orally administrable composition is prepared as follows:

30 β-carotene (Roche) 4.7 mg

Zeaxanthine 10 mg

Latobacillus CNCM I-1225 dry mix as in example 1 50 mg
Glucidex IT 19 (maltodextrin powder) QSP 500 mg

The composition is administered to the individual in an amount of 2x500 mg daily,
5 which provides a protective and preventive effect of the skin.

• **Example 3**

A photoprotective daily orally administrable composition is prepared as follows:

10 β -carotene (Roche) 4.7 mg
Lycopene (Lycored) 2.5 mg
Bifidobacterium CNCM I-2168 (containing 1.10^8 to 1.10^9 organisms) 30 mg
Latobacillus CNCM I-1225 dry mix as in example 1 30 mg
Glucidex IT 19 (maltodextrin powder) QSP 500 mg

15 The composition is administered to the individual in an amount of 2x500 mg daily,
which provides a protective and preventive effect of the skin.

Example 4

20 A photoprotective daily orally administrable composition is prepared as follows:

Lycopene (Lycored) 2.5 mg
Lyophilized *S.cerevissae* (BioSpringer) 75 mg
Latobacillus CNCM I-2116 dry mix (containing 1.10^8 to 1.10^9 organisms) 50 mg
25 Glucidex IT 19 (maltodextrin powder) QSP 500 mg

The composition is administered to the individual in an amount of 2x500 mg daily,
which provides a protective and preventive effect of the skin.

30 **Example 5**

A photoprotective daily orally administrable composition is prepared as follows:

	β -carotene	(Roche)	4.7 mg
	Lycopene	(Lycored)	2.5 mg
	Lyophilized <i>S.cerevissae</i>	(BioSpringer)	75 mg
5	Latobacillus CNCM I-1225 dry mix as in example 1		50 mg
	Glucidex IT 19 (maltodextrin powder)	QSP 500 mg	

The composition is administered to the individual in an amount of 2x500 mg daily, which provides a protective and preventive effect of the skin.

10

Example 6

A photoprotective daily orally administrable composition is prepared as follows:

	β -carotene	(Roche)	4.7 mg
15	Lyophilized <i>S.cerevissae</i>	(BioSpringer)	75 mg
	Latobacillus CNCM I-1225 dry mix as in example 1		50 mg
	Glucidex IT 19 (maltodextrin powder)	QSP 500 mg	

20

The composition is administered to the individual in an amount of 2x500 mg daily, which provides a protective and preventive effect of the skin.

21.02.2002

(43)

Claims

1. An orally administrable composition for the photoprotection of the skin which comprises a photoprotecting effective amount of i) at least one probiotic lactic acid bacterium or a culture supernatant thereof, and ii) at least one carotenoid or derivative, included into an orally acceptable carrier.
5
2. A composition according to claim 1, in which the lactic acid bacterium is selected from the group consisting of Lactic acid bacteria, preferably Lactobacilli and/or Bifidobacteria.
10
3. A composition according to claim 1 or 2, in which the lactic acid bacterium is *Lactobacillus johnsonii*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Lactobacillus casei* or *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium animalis*, *Bifidobacterium infantis*, *Bifidobacterium adolescentis*, *Bifidobacterium pseudocatenulatum*, or a mixture thereof.
15
4. A composition according to one of claims 1 to 3, in which the lactic acid bacterium is CNCM I-1225, CNCM I-2116, CNCM I-2168 or CNCM I-2170.
20
5. A composition according to one of claims 1 to 4, in which the probiotic lactic acid bacterium is included into the carrier in a live form, semi-active or in deactivated form, preferably as a lyophilized powder attention.
25
6. A composition according to one of claims 1 to 5, wherein the carotenoid is a carotenoid with or without provitamin A activity, such as β -carotene, γ -carotene, α -carotene, lycopene, zeaxanthine and luteine, or a mixture thereof.
30
7. A composition according to one of claims 1 to 6, wherein the carrier is a food or a pharmaceutical product, or a nutritional supplement for oral administration.

8. A composition according to claim 6, wherein the food or pharmaceuticals carriers are milk, yoghurt, curd, cheese, fermented milks, milk based fermented products, ice-creams, fermented cereal based products, milk based powders, infant formulae or tablets, liquid suspensions, dried oral supplement, wet oral supplement, dry-tube-feeding.
9. A composition according to claim 7, wherein the nutritional supplement for oral administration may be in capsules, soft capsules, tablets, pastes or pastilles, gums, or drinkable solutions or emulsions.
10. A composition according to one of claim 1 to 9, which further comprises a yeast extract or a bioactive molecule.
11. Use of a photoprotecting effective amount of at least one probiotic lactic acid bacterium or a culture supernatant thereof and at least one carotenoid, included into an orally acceptable carrier, for preparing an orally administrable composition for the protection of the skin against solar radiations and attenuating or preventing all related skin disorders.
12. The use according to claim 11, wherein the probiotic lactic acid bacterium is present in the carrier in an amount of from about 10^5 to 10^{12} cfu/ g carrier.
13. The use according to claim 11, wherein the carotenoid is present in the carrier in an amount of from $10^{-12}\%$ to 20% by weight.
14. A method for improving the photoprotective function of the skin, which comprises the step of orally administering to the individual a composition comprising the combination of i) at least one probiotic lactic acid bacteria or a culture supernatant thereof, and ii) at least one carotenoid or derivative, in an orally acceptable carrier.

EPO - DG 1

12

21 02. 2002

Abstract

(43)

Orally administrable composition for the photoprotection of the skin

- 5 An orally administrable composition for the photoprotection of the skin which comprises the combination of i) at least one probiotic lactic acid bacterium or a culture supernatant thereof, and ii) at least one carotenoid or derivative, included into an orally acceptable carrier.

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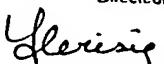
DT09 Rec'd PCT/PTO 20 AUG 2004

I. IDENTIFICATION DU MICRO-ORGANISME	
Référence d'identification donnée par le DEPOSANT :	Numéro d'ordre attribué par l'AUTORITE DE DEPOT INTERNATIONALE :
La 1	I - 1225
II. DESCRIPTION SCIENTIFIQUE ET/OU DESIGNATION TAXONOMIQUE PROPOSEE	
Le micro-organisme identifié sous chiffre I était accompagné :	
<input checked="" type="checkbox"/> d'une description scientifique	
<input checked="" type="checkbox"/> d'une désignation taxonomique proposée	
(Cocher ce qui convient)	
III. RECEPTION ET ACCEPTATION	
La présente autorité de dépôt internationale accepte le micro-organisme identifié sous chiffre I, qu'elle a reçu le 30.06.1992 (date du dépôt initial)	
IV. RECEPTION D'UNE REQUETE EN CONVERSION	
La présente autorité de dépôt internationale a reçu le micro-organisme identifié sous chiffre I le _____ (date du dépôt initial) et a reçu une requête en conversion du dépôt initial en dépôt conforme au Traité de Budapest le _____ (date de réception de la requête en conversion)	
V. AUTORITE DE DEPOT INTERNATIONALE	
Nom : Collection Nationale de Cultures de Microorganismes Institut Pasteur 25, Rue du Docteur Roux Adresse : 75724 PARIS CEDEX 15	Signature(s) de la (des) personne(s) compétente(s) pour représenter l'autorité de dépôt internationale ou de l'(des) employé(s) autorisé(s) Date : Paris le 02 Juillet 1992 Mme Y. CERISIER <i>Y. Cerisier</i>

10/505305

PCT/EP03/01685

DTOS Rec'd PCT/PTO 20 AUG 2004

I. IDENTIFICATION DU MICRO-ORGANISME	
Référence d'identification donnée par le DEPOSANT : NCC 2461	Numéro d'ordre attribué par l'AUTORITE DE DEPOT INTERNATIONALE : I.- 2116
II. DESCRIPTION SCIENTIFIQUE ET/OU DESIGNATION TAXONOMIQUE PROPOSEE	
Le micro-organisme identifié sous chiffre I était accompagné : <input checked="" type="checkbox"/> d'une description scientifique <input checked="" type="checkbox"/> d'une désignation taxonomique proposée (Cocher ce qui convient)	
III. RECEPTION ET ACCEPTATION	
La présente autorité de dépôt internationale accepte le micro-organisme identifié sous chiffre I, qu'elle a reçu le 12 JANVIER 1999 (date du dépôt initial)	
IV. RECEPTION D'UNE REQUETE EN CONVERSION	
La présente autorité de dépôt internationale a reçu le micro-organisme identifié sous chiffre I le 12 JANVIER 1999 (date du dépôt initial) et a reçu une requête en conversion du dépôt initial en dépôt conforme au Traité de Budapest le 12 FÉVRIER 1999 (date de réception de la requête en conversion)	
V. AUTORITE DE DEPOT INTERNATIONALE	
Nom : CNCM Collection Nationale de Cultures de Microorganismes	Signature(s) de la (des) personne(s) compétente(s) pour représenter l'autorité de dépôt internationale ou de l'(des) employé(s) autorisé(s) : Mme Y. CERISIER Directeur Administratif de la CNCM
Adresse : INSTITUT PASTEUR 28, Rue du Docteur Roux F-75724 PARIS CEDEX 15	 Date : Paris, le 12 février 1999

10/505305

PCT/EP03/01685

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I. IDENTIFICATION DU MICRO-ORGANISME	
Référence d'identification donnée par le DEPOSANT :	Numéro d'ordre attribué par l'AUTORITE DE DEPOT INTERNATIONALE :
NCC 251	I - 2168
II. DESCRIPTION SCIENTIFIQUE ET/OU DESIGNATION TAXONOMIQUE PROPOSEE	
Le micro-organisme identifié sous chiffre I était accompagné :	
<input checked="" type="checkbox"/> d'une description scientifique	
<input checked="" type="checkbox"/> d'une désignation taxonomique proposée	
(Cocher ce qui convient)	
III. RECEPTION ET ACCEPTATION	
La présente autorité de dépôt internationale accepte le micro-organisme identifié sous chiffre I, qu'elle a reçu le 15 MARS 1999 (date du dépôt initial)	
IV. RECEPTION D'UNE REQUETE EN CONVERSION	
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V. AUTORITE DE DEPOT INTERNATIONALE	
Nom : CNCM Collection Nationale de Cultures de Microorganismes	Signature(s) de la (des) personne(s) compétente(s) pour représenter l'autorité de dépôt internationale ou de l'(des) employé(s) autorisé(s) : Simona OZDEN Directrice de la CNCM 
Adresse : INSTITUT PASTEUR 28, Rue du Docteur Roux F-75724 PARIS CEDEX 15	Date : Paris, le 10 août 1999 

I. IDENTIFICATION DU MICRO-ORGANISME	
Référence d'identification donnée par le DEPOSANT :	Numéro d'ordre attribué par l'AUTORITE DE DEPOT INTERNATIONALE :
NCC 490	I - 2170
II. DESCRIPTION SCIENTIFIQUE ET/OU DESIGNATION TAXONOMIQUE PROPOSEE	
Le micro-organisme identifié sous chiffre I était accompagné :	
<input type="checkbox"/> d'une description scientifique.	<input type="checkbox"/> d'une désignation taxonomique proposée
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III. RECEPTION ET ACCEPTATION	
La présente autorité de dépôt internationale accepte le micro-organisme identifié sous chiffre I, qu'elle a reçu le 15 MARS 1999 (date du dépôt initial)	
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V. AUTORITE DE DEPOT INTERNATIONALE	
Nom : Adresse :	<p>CNCM Collection Nationale de Cultures de Microorganismes</p> <p>INSTITUT PASTEUR 28, Rue du Docteur Roux F-75724 PARIS CEDEX 15</p> <p>Signature(s) de la (des) personne(s) compétente(s) pour représenter l'autorité de dépôt internationale ou de l'(des) employé(s) autorisé(s) : Simona OZDEN Directeur de la CNCM</p> <p>Ozden</p> <p>Date : Paris, le 10 août 1999</p>

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